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Bone Marrow Histology Characteristics in MPL^{515} Mutated Thrombocythemia with Various Degrees of Myelofibrosis: A Cross Sectional Follow-up Study in Eight Cases

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Abstract

MPL$^{W515l/K}$ mutated Essential Thrombocythemia (ET) usually present with increased platelet counts around 1000 x 10^9/L as the only abnormal laboratory finding with normal values for hemoglobin and leukocytes and no or minor splenomegaly on palpation. Early stage MPL^{515} mutated ET show the presence of clustered small and giant megakaryocytes with pronounced deeply lobulated nuclei, which are not seen in JAK2V617F positive ET, prodromal PV, and classical PV. MPL^{515} mutated ET has no clinical, laboratory and bone marrow features of prodromal PV. Clustering of large mature large to giant megakaryocytes with pronounced hyper lobulated nuclei in a normocellular bone marrow is the hallmark of JAK2-wild type MPL^{515} mutated thrombocythemia. Bone marrow histology in MPL^{515} mutant patients revealed isolated megakaryocytic proliferation in a normocellular bone marrow at diagnosis with a reduction of erythropoiesis during follow-up. MPL^{515} Thrombocythemia do not have PV features at diagnosis, do not evolve into PV, have normal LAP score, serum EPO and ferritin levels. In contrast to JAK2^{V617F} positive ET, no spontaneous Endogenous Erythroid Colonies (EEC) was found in none of evaluated MPLW5^{15}L cases. Spontaneous megakaryocyte growth in culture with an overall normal response to ThromboPoietin (TPO) has been demonstrated in two MPL^{515} mutated cases.

Keywords: Hemoglobin; Leukocytes; Nuclei; Bone marrow; Diagnosis

Introduction

Animal models overexpressing c-MPL in transgenic mice manifested with typical features of ET with a four-fold increase of platelet count, increased colony formation of megakaryocytes, and increase of clustered enlarged megakaryocytes in the bone marrow [1,2]. The ET animals appeared healthy, had a very slight decrease of hematocrit from 0.42 to 0.39 in control and MPL mice respectively and survived normally with no evidence of myelofibrosis in the bone marrow. The first case of congenital ET by Ding et al in 2004 in a Japanese family caused by the germline MPLS505N mutation [3]. In 2006, two novel MPL515 somatic mutations (MPLW515L and MPLW515K) have been discovered in 5% and 1% in large cohorts of acquired ET and Myelofibrosis (MF) patients respectively [4,5]. Within one center cohort of 402 MPN patients in Seoul there were 3 cases of ET and 3 cases of myelofibrosis (MF) patients (N=6, 1.7% of 402, cases 2 to 7, Table 1) who carry an acquired gain of function mutation of the MPL receptor as the cause of ET or MF [6]. MPN515 case 1 is the first one found in our cohort of ET patients seen by the MPN study group in Rotterdam Antwerp and Brussels, who carry an acquired gain of function mutation of the MPL receptor as the cause of ET. MPN515 case 8 is the first one found in a large cohort of ET patients seen by the MPN study group in Prague by Campr & Schwarz.

Case/Age	Hb	Hct	RBC	WBC	Platelet	EPO	LDH	Spleen	BM	Date
Gender	g/dL		10^{12}/l	10^9/l	10^9/l	U/mL	Units	Size cm		
1. 73 F	12.5	0.39	4.2	6.9	1243	nt		13 cm	ET	01 01 2015
2. 69 F	11.3	0.34	3.6	7.8	678	1.93		normal	ET	13 03 2012
3. 66 M	13.1	39.8		8.2	802		689	11 cm	ET	01 01 2011
4. 54 M	10.6	0.33		19.6	1290		625	increased	ET/MF	01 05 2004
4. 60 M	6.9	0.21		3.7	342			large	MF 3 RF 4	01 03 2010

5. 78 M	8.9	0.28		5.1	252	0.85	1618	17 cm	MF 3 RF 4	01 08 2011
6. 48 F	5.4	0.16	1.8	4.4	166			28 cm	MF 3 RF 4	ABMT 2006
6. 51 F	5.4	0.165		blasts	186				Fig	AML
7. 69 F	7.9	0.26		2.2	1256	1.08	388	24 cm	ET/MF	01 09 2012
7. 71 F	pancytopenia			3.6 blasts	85				AML	01 04 2014
8. 41 F	12.8	0.39		7.5	790		315	12 cm	ET	01 01 1996

Table 1: Laboratory data in eight cases of MPL^{515} mutated normocellular Essential Thrombocythemia (ET) and ET with various degrees of Myelofibrosis (MF).

Bone Marrow Histopathology Method

As a main prerequisite for bone marrow diagnosis of myeloproliferative neoplasms professionally performed representative biopsies from the iliac crest with an orthograde direction of the trephine are warranted in MPLW515l/K mutated ET [7,8]. Fixation of the specimens is usually carried out in an aldehyde solution of low concentration (2%-3%), or preferentially, in a mixture containing 2 ml of 25% glutaraldehyde, 3ml of 37% formaldehyde, 1.58 g anhydrous calcium acetate and distilled water per 100 ml. For achievement of optimal quality for enzyme- and or immunochemistry, any acid medium has to be avoided, including so-called acid fast decalcifying solutions. The next step normally consists of paraffin embedded and employment of several staining techniques routinely involving Giemsa, Hematoxylin and Eosin (HE), PAS (Periodic Acid Schiff reagent), nathptol-AS-D-chloroacetate esterase, Perls'reaction for iron and silver impregnation method, following Gomori's techniques. For a specific staining of marrow cells, a number of monoclonal antibodies have been recommended: CD61 (antiplatelet Glycoprotein IIIa) or CD66 for the identifaction of megakaryocytes including precursors (promegakaryoblasts and megakarypblasts) and CD71 to stain selectively erythropoiesis. Bone marrow histology features of megakaryocytes including their number, size, morphology and clustering are the diagnostic clue to each of the early and advanced stages of the MPNs subtype of megakaryocytic myeloproliferation and secondary myelofibrosis related to various degrees of increased/decreased erythropoiesis and/or granulopoiesis. The quality (reticulin - collagen) and pattern of density of the fiber content can contribute significantly to defining the MPN stage in each patient at time of first diagnosis for the purpose of prognostic prediction. All these features have been explicitly described by the ECP and ECMP criteria for the classification of three distinct myeloproliferative disorders of ET, Polycythemia Vera (PV) and Primary Megakaryocytic Granulocytic Myeloproliferation (PMGM).

Clinical Cases

Case 1: An isolated high platelet count of 1243 × 10^9/L was found in an asymptomatic 73-year-old woman during a routine laboratory diagnostic work-up for hypertension. Laboratory features at time of diagnosis were, hemoglobin 12.5 g/L, hematocrit 0.39, erythrocytes 4.2 × 10^{12}/L, MCV 82 fL, leukocytes 6.9 × 10^9/L, normal LDH and spleen size on echogram 12.6 cm (normal value <12 cm). Bone marrow histology findings diagnostic for MPL515 ET in a normocellular bone marrow are described in great detail in Figures 1 to 4.

Case2: A 69-year old woman born in 1942 was referred with anemia for routine examination. Laboratory features at time of diagnosis were, hemoglobin 11.3 g/dL, hematocrit 0.33, leukocyte 7.8 x10^9/L, platelet 678 x10^9/L, LDH 458 IU/L. Bone marrow biopsy revealed megakaryocytic hyperplasia which were consistent with MPLW515 mutated ET (Figure 5).

Case 3: A 66-year old man visited emergency room because of an atypical cerebral ischemic attack dysarthria (Jan 2011). Laboratory features at time of diagnosis were, hemoglobin 13.1 g/dL, hematocrit 39.8%, leukocyte 8.24×10^9/L, platelet 802×10^9/L, LDH 689 IU/L, and no splenomegaly (11.3cm length diameter on echogram). Bone marrow histology in (Figure 6) showed a cellularity of about 50% with marked megakaryocytosis consistent with normocellular ET without features of PV similar as in case 2. Hydroxyurea and aspirin were maintained for 4 years and patient remained asymptomatic and well during follow-up until November 2014.

Case 4: A 54-year old man born in 1949 presented in May 2004 with MPLW515 mutated ET complicated by painful left 2-4th fingertips and left toe tip and right foot tingling sensation (erythromelalgia). Laboratory features at time of ET diagnosis were, hemoglobin 10.6 g/dL, hematocrit 33.1%, leukocyte 19.63 × 10^9/L, platelet 1290 × 10^9/L, LDH 625 IU/L, and splenomegaly. Bone marrow histology revealed increased cellularity of about 60% with marked megakaryopoiesis of large to giant megakaryocytes with hyperlobulated nuclei consistent with ET without features of PV. Hydroxyurea and aspirin were prescribed and he was follow-up loss after June 2005. In March 2010 at age 61, he had developed a transfusion dependent anemia and significant splenomegaly on abdominal MRI. Laboratory findings at this time were as follows; Hemoglobin 6.9 g/dL, hematocrit 21.2%, leukocyte 3.70 × 10^9/L, platelet 342 × 10^9/L. Bone marrow showed a hypercellular marrow with diffuse fibrosis. The clinical diagnosis was hydroxyurea-induced myelofibrosis IPSS Int-2. He refused active treatment except blood transfusion.

Case 5: A 78-year old man first visited our hospital because of dizziness, anemia and splenomegaly in August 2011. Laboratory features at time of diagnosis were, hemoglobin 8.9 g/dL, hematocrit 27.6%, leukocyte 5.13×10^9/L, platelet 252×10^9/L, LDH 1618 IU/L, and splenomegaly (17cm length diameter on echogram). Bone marrow pathology revealed packed marrow with diffuse fibrosis (RF grade 3) consistent with Myelofibrosis (MF). Danazol 400 mg was maintained and he was followed until January 2015.

Case 6: A 48-year old woman born in 1961 first presented in April 2005 with severe anemia and MPL515 mutated MF hemoglobin 5.4 g/dL, hematocrit 0.16, erythrocytes 1.78 × 10^{12}/L, leukocytes 4.4

$\times 10^9$/L, platelets 166 $\times$ 10^9/L. Splenectomy of a large spleen (27, 9 cm length diameter) was performed in January 2006 and portal and splenic vein thrombosis were detected. Bone marrow histology showed tightly clustered large dysmorphic megakaryocytes with hyperchromatic nuclei with decreased erythropoiesis and increased reticulin fibrosis grade 3. Allogeneic HSCT in June 2006 of a matched unrelated donor and non-myeloablative conditioning (Busulfan-Fludarabine-TBI) was followed by rapid engraftment and complete hematological remission. Slow onset anemia aggravated in October 2010 (hemoglobin 5.4 g/dL, hematocrit 16.5%, leukocyte 4.4 x 10^9/L, platelets 186 $\times$ 10^9/L) and bone marrow pathology revealed a 30% cellularity and diffuse fibrosis. Patient had been treated with c-HUR for 2 years and followed up until January 2013.

Case 7: A 69-year old woman born in 1942 first presented with left abdominal discomfort pain due to splenomegaly (spleen size 24 cm length diameter) in September 2012 with MPL515 mutated ET. Laboratory features at time of diagnosis were, hemoglobin 7.9 g/dL, hematocrit 0.26, leukocyte 2.2 $\times 10^9$/L, platelet 1256 $\times$ 10^9/L, LDH 388 IU/ L. Bone marrow revealed decreased cellularity (50%) with increase of small immature dysmorphic megakaryocytes consistent with myelodysplastic transformation (Figure 8). She had been treated with erythropoietin, aspirin and hydroxyurea and intermittent red cell transfusion. Bone marrow biopsy in April 2014 because of increased blasts in peripheral blood (leukocyte 3.63$\times 10^9$ / L and 13% blasts) showed reticulin fibrosis and 100% cellularity with blasts of about 12% of nucleated elements. Chromosomal abnormality was detected as 47,XX,+8[15] / 46,XX[5]. Synthetic steroid ethisterone (danazol) and anagrelide (agrylinR) were started. Fever was developed Jun 2014 and pancytopenia was detected (hemoglobin 8.8 g/dL, hematocrit 26.2%, leukocyte 3.08$\times 10^9$/L, platelet 85$\times 10^9$/L). Pneumonia was aggravated and patient died 3 days after admission.

Case 8: Schwarz and Campr described the natural history of a 41-year old woman born in 1945 who presented in January 1996 with MPLW515L mutated ET [7]. Clinical history consisted of a one year history of tingling prickling sensations in fingers and hand, vertigo and attacks of frontal headaches at platelet counts of 790 $\times 10^9$/L. Laboratory features at time of diagnosis were, hemoglobin 12.8 g/L, hematocrit 0.39, leukocytes 7.5$\times 10^9$/L, platelets 790 $\times$109/L with highest platelet count of 1996$\times 10^9$/L in October 1996, normal LDH and spleen size on echogram 12.6 cm (normal value <12 cm). Molecular biology analysis were negative for the JAK2V617F, CALR and ASXL1 mutations. Bone marrow histology showed a normal cellularity of about 40%, no increase of erythropoiesis, and prominent increase of large to giant megakaryocytes with hyperlobulated nuclei even stag-horn forms with fine chromatin (Figure 9). Sporadically, small megakaryocytes with less lobulated nuclei were present. Fine perivascular Reticulin Fibers (RF grade 1) consistent with pre-fibrotic Myelofibrosis (MF). Treatment consisted of low dose aspirin for the relief of microvascular disturbances, pegylated interferon (IFN) was not tolerated and subsequent treatment consisted of hydroxyurea from 1996 to 1999 was followed by anagrelide from January 1999 untill she developed refractory pancytopenic anemia and thrombocytopenia in 2004 at age of 59 years. Bone marrow histology in 2004 showed a hypocellular bone marrow with dysmorphic small to large megakaryocytes and only slight increase of reticulin fibers RF grade 1 to 2. Hematopioetic Stem Cell Transplantation (HSCT) in February 2005 of a matched (10/10) unrelated donor and non-myeloablative conditioning (Busulfan-Cytarabine-ATG) was followed by rapid engraftment and complete hematological remission. She is more than 10 years alive and well in 2015 and beyond

Results

Bone marrow histology in MPL mutated thrombocythemia and myelofibrosis in case 1 are shown in great detail in figures 1 to 4) and featured by slight increased cellularity and loosely clustered large to giant megakaryoctyes with pronounced hypersegmented nuclei with no increase in erythropoiesis or granulopoiesis. There was slight increase in reticuline fibers without crossing-overs. Bone marrow histology in case 2 (Figure 5) show normocellular bone marrow with loosely clustered larged megakaryoctyes with hypersegmented nuclei with no increase in erythropoiesis or granulopoiesis and slight increase in reticuline fibers with a few crossing-overs. Bone marrow histology in case 3 (Figure 6) show normocellular bone marrow, medium-sized megakaryoctyes, no clustering of megakaryocytes, decrease in erythropoiesis, normal granulopoiesis and no increase in reticuline fibers. Bone marrow histology in case 6 (Figure 7) show tightly clustered large dysmorphic megakaryocytes with hyperchromatic nuclei and some megakaryocytes with naked nuclei. The overall cellularity is increased with decreased erythropoiesis and increased reticulin fibrosis grade 3. Bone marrow histology in case 7 show slightly increased cellularity, normal-sized megakaryocytes with sometimes a hypolobulated nucleus, and there is maturation-arrest of the granulopoiesis with decreased granulopoiesis consistent with MPL515 mutated MPN in transformation to MDS (Figure 8). Bone marrow histology in case 8 at time of MPL515 thrombocythemia presentation in figure 9 shows a normocellular bone marrow, loosely clustered larged megakaryoctyes with hypersegmented nuclei, no increase in erythropoiesis or granulopoiesis and no increase in reticuline fibers. The bone marrow histology in case 8 progressed into refractory anemia in 2004 as demonstrated by decrease in cellularity, large dysmorphic megakaryocytes and minor increase in reticulin fibers without crossing-overs (data not shown).

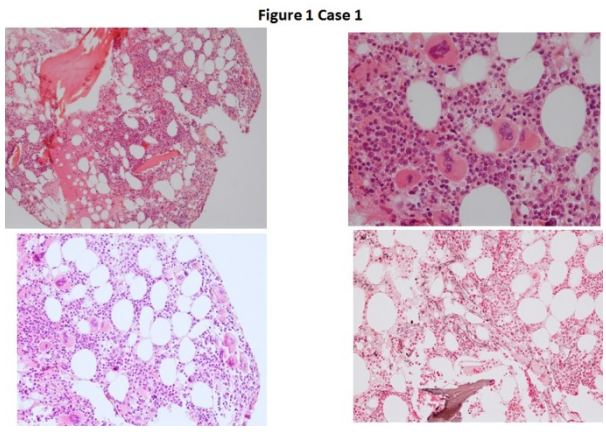

Figure 1: Standardized set of bone marrow histology pictures in MPL515 mutated thrombocythemia in case 1 showing slight increase of reticulin fibrosis, slight increased cellularity and loosely clustered large to giant megakaryoctyes with hypersegmented nuclei, and normal erythropoiesis and granulopoiesis. Slight increase in reticuline fibers without crossing-overs (RF grade 1).

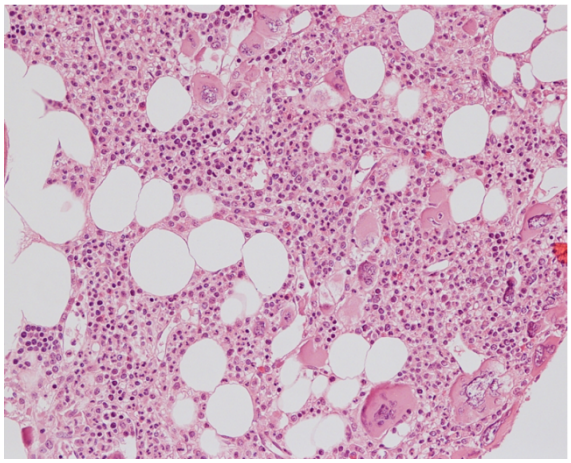

Figure 2: Detail of bone marrow histology in MPL^{515} case 1: normocellular bone marrow. Loosely clustered larged megakaryoctyes with hypersegmented nuclei. No increase in erythropoiesis or granulopoiesis

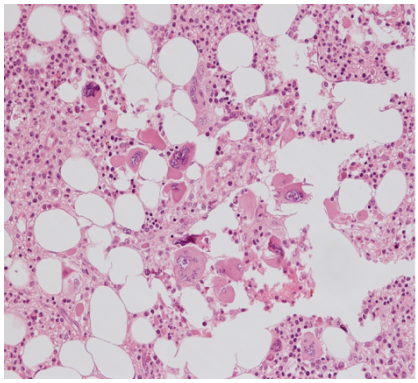

Figure 3: Another detail of bone marrow histology in MPL515 case 1 : normocellular bone marrow. Loosely clustered larged megakaryoctyes with hypersegmented nuclei. No increase in erythropoiesis or granulopoiesis.

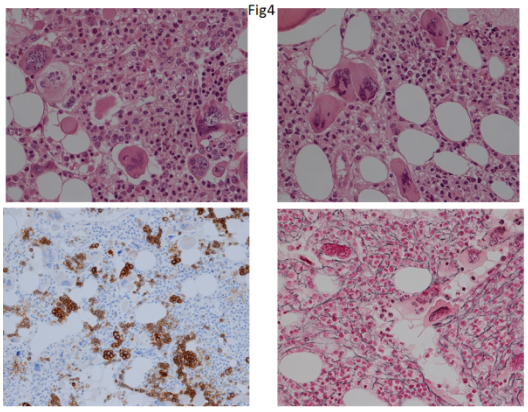

Figure 4: Another standardized set of bone marrow histology pictures in MPL515 case 1 showing normocellular bone marrow with medium to large sized megakaryoctyes. No clustering. Normal erythropoiesis (CD-71 stain). Normal granulopoiesis. Slight increase in reticuline fibers (RF) with a few crossing-overs: RF grade ½

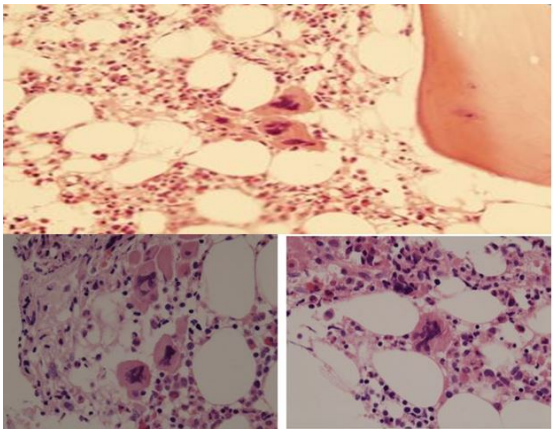

Figure 5: Routine bone marrow histology in MPL515 case 2: tightly clustered large dysmorphic megakaryocytes with hyperchromatic nuclei. Some megakaryocytes with naked nuclei. Increased cellularity. Decreased erythropoiesis. Reticulin fibrosis grade 1.

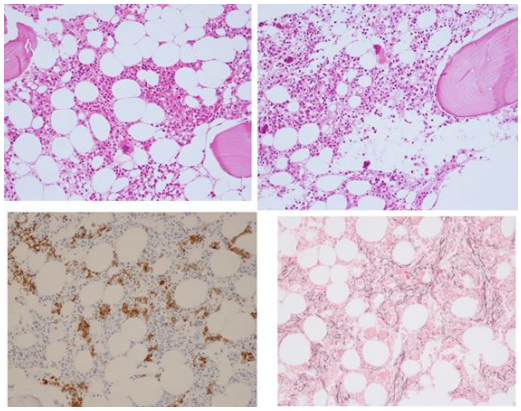

Figure 6: Standardized set of bone marrow histology pictures in MPL515 case 3: tightly clustered large dysmorphic megakaryocytes with hyperchromatic nuclei. Some megakaryocytes with naked nuclei. Slight increased cellularity. Normal erythropoiesis (CD-71 stain). Reticulin fibrosis grade 2.

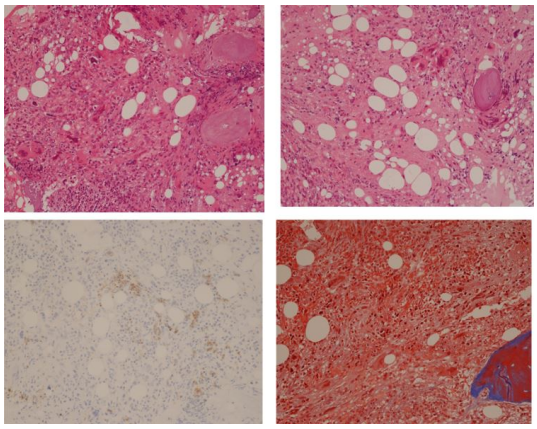

Figure 7: Bone marrow histology findings in MPL515 case 6 showing increased cellularity and increased fibrosis grade 3. Normal-sized megakaryocytes with sometimes a hypolobulated nucleus. Maturation-arrest of the granulopoiesis. Strongly decreased erythropoiesis (CD-71 stain). MPL515 mutated MPN in myelofibrotic transformation.

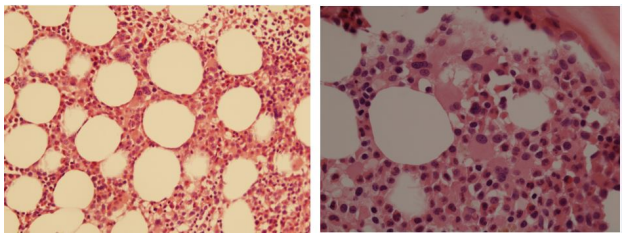

Figure 8: Bone marrow histology findings in MPL515 case 7 at time of progression of MPL515 MPN disease into refractory anemia with myelodysplastic features of decreased cellularity and small dysmorphic megakaryocytes. Minor increase in reticulin fibers.

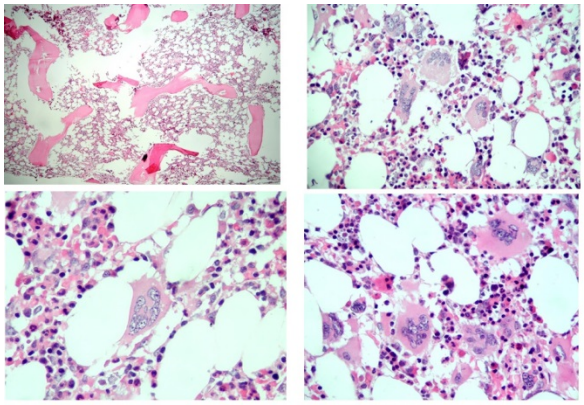

Figure 9: Bone marrow histolology findings in MPL515 case 8 featured by increase of large to giant megakaryocytes with pronounced hyperlobulated nuclei and slight reduction of erythropoiesis consistent with the diagnosis of MPL515 mutated essential thrombocythemia (ET).

Discussion

The frequency of the MPLW515L/K mutation in the original studies were 5.3% in the JAK2V617F wild type (WT) ET and 9.6% in JAK2V617F wild type PMF patients [4,5]. The rare occurrence rate of the MPLW515L/K mutations have been confirmed in two recent studies within large groups of WHO defined MPN population and JAK2 WT ET and MF population [6-9]. The Italian GIMEMA cross sectional study subdivided 952 ET patients into 546 JAK2V617F mutated (57%) and 418 JAK2 wild type (43%) and found 30 cases (3% of total ET and 7.2% of JAK2 wild type ET) carrying the MPLW515WL/K mutation. MPLW515L/K and JAK2V617F coexisted in 3 patients with MPLW515L and in 5 with MPLW515K allele [9]. Microvascular disturbances were recorded equally high in 31% of JAK2V617F ET and in 25% of JAK2/MPL wild type ET (in retrospect mainly CALR mutated). Increased platelet counts of 956 + 331x10^9/L was the only abnormal laboratory finding in MPLW515l/K mutated ET with normal values for hemoglobin (13.4 + 1.3 g/l) and leukocytes (8.8 + 3.1x10^9/L). There was slight increase of LDH (459+182 U/L). Splenomegaly on palpation was only present in 5 (17%) of 30 MPL^{515} mutated ET cases as compared to 21% in JAK2V617F mutated ET and 20% in JAK2/MPL wild type ET (in retrospect mainly CALR mutated)

[9]. A previous bone marrow histology study by Michiels et al. in 12 out of these 30 Italian MPL^{515} mutated ET patients showed main differences in bone marrow histopathology between patients with MPL^{515} mutated (N=12) versus JAK2V617F mutated MPN [10]. Early stage MPL^{515} mutated ET show the presence of clustered small and giant megakaryocytes with pronounced deeply lobulated nuclei, which are not seen in JAK2V617F positive ET, prodromal PV, and classical PV [10] indicating that MPL^{515} mutated ET have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis, do not evolve into PV during follow-up [10], and have normal LAP score, serum EPO and ferritin levels. In the present study we described in great detail bone marrow histology of MPL^{515} mutated thrombocythemia with high platelet count of 1243x10^9/L in an asymptomatic 73-year-old woman (Figure 1 to 4) [11]. Laboratory features at time of diagnosis were, hemoglobin 12.5 g/L, hematocrit 0.39, erythrocytes 4.2x10^{12}/L, MCV 82 fL, leukocytes 6.9x10^9/L, normal LDH and spleen size on echogram 12.6 cm (normal value <12 cm). This case of acquired MPL515 mutated thrombocythemia typically show large to giant mature megakaryocytes with pronounced hyperlobulated nuclei in a completely normocellular bone marrow with no increase of erythropoiesis and minor increase of reticulin fibers (RF 1) (Figure 4). Both erythroid and granulocytic cellularity were reduced in cases 2 and 3 of MPL515 mutated ET indicating the absence of PV features. As shown in Figure 1 to 4 and 11, clustering of large mature large to giant megakaryocytes with hyperlobulated in a normocllular bone marrow appears to be the hallmark of acquired JAK2-wild type MPL^{515} mutated thrombocythemia. Overall, bone marrow histology in MPL^{515} mutant patients revealed more isolated megakaryocytic proliferation in a normocellular bone marrow at diagnosis with a pronounced or reduction of erythropoiesis in figures 5 and 8 respectively. Beer et al described bone marrow biopsies at diagnosis of 13 patients with MPL mutations: MPLS505N in two, MPLW515K in two, MPLW515L in nine patients [12]. As compared to JAK2V617F positive ET and JAK2/MPL wild type ET, the bone marrow biopsies from the MPL515 mutant MPN were less cellular (P<.001 and P<.003 with age). In contrast to JAK2V617F positive ET, no spontaneous endogenous erythroid colonies (EEC) was found in any of evaluated MPLW515L cases (4 ET and 1 MF) in two studies [12,13]. Spontaneous megakaryocyte growth in culture with an overall normal response to Thrombopoietin (TPO) has been demonstrated in two MPL mutated cases, but the erythroid progenitors remained EPO dependent and did not show spontaneous erythroid colony (EEC) formation [13]. We conclude that the present and previous studies clearly demonstrate that the megakaryocytes in early stage MPL^{515} mutated ET are large to giant with pronounced hyperlobulated nuclei in a normocellular bone marrow with normal or reduced erythropoiesis [10-14]. Such large and giant megakaryocytes with pronounced hyperlobulated nuclei were rare in JAK2V617F ET patients in the studies of Piche et al. [11] and Michiels et al. [14].

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